

REMARKS

Reconsideration of this application is requested. Claims 1-8 will be active in the application subsequent to entry of this Amendment.

The issues raised in the outstanding Official Action are addressed in the order presented.

Attached is a Terminal Disclaimer relating to U.S. 5,639,672 which responds to items 1 and 2 of the Official Action and resolves the obviousness-type double patenting rejection specifically set out in item 2.

Page 6 of the specification has been amended in order to address the examiner's comments in item 3 of the Official Action. The adjustments made to page 6 agree with the more detailed description found on page 15, lines 6-21 of the specification; no new matter is involved.

Claim 1 has been amended in order to more particularly point out and distinctly claim that which applicants regard as their invention and to address the examiner's comments in items 4 and 5 of the Official Action. Claim 1 now recites the specific steps of the method, as the examiner suggested, and relates these steps to the overall process. In addition, the formazan produced in the detection method is defined as water-soluble. This is based upon the description of the invention found on page 11, lines 13-20 of the specification and results from the use of a tetrazolium salt which produces a water-soluble and chemically stable formazan.

New claims 6 and 7, dependent from claims 1 and 4, respectively, have been added directed to a specific tetrazolium salt preferred by applicants and disclosed on page 11, lines 14-16 of the specification (and elsewhere). Claim 8 is directed to the embodiments of the inventions disclosed on page 12 of the specification.

Applicants submit that the claims now under review are compliant with 35 U.S.C. § 112, second paragraph. Reconsideration is requested.

In items 6-7 of the Official Action, claims 4 and 5 are rejected as allegedly being anticipated by Burd et al. Distinct differences exist between the biosensor recited in

claims 4 and 5 of the present application and Burd et al U.S. Patent 5,639,672, hence there is no anticipation.

Burd et al describes NBT (nitroblue tetrazolium), but its Examples describe only the results of measurement by direct electrolysis of fructosamine. The inventors of the present invention previously investigated specific oxidation peak potentials for this NBT and other tetrazolium salts by cyclic voltammetry, but found no specific oxidation peak potential in the range of from -1.0 V (vs. Ag/AgCl) to $+1.0$ V (vs. Ag/AgCl).

According to Burd et al, an oxidation potential of $+1.04$ V (vs. Ag/AgCl) was applied for the direct electrolysis of fructosamine. On the other hand, the present inventors have confirmed that upon application of a high potential of $+0.7$ V (vs. Ag/AgCl) or more, measurement is greatly affected by the various coexisting materials contained in the sample.

By contrast, in the present invention, the use of WST-1 (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt provides a highly sensitive biosensor which detects a specific oxidation peak potential at about $+0.5$ V (vs. Ag/AgCl) and is minimally influenced by extraneous matter in the sample.

Furthermore, the tetrazolium salt used in Burd et al is a sparingly water-soluble tetrazolium salt. Thus, it is too problematical in solubility to be used as a convenient, reliable biosensor. The present invention uses WST-1, a water-soluble tetrazolium salt, which has high solubility and can be effectively and accurately used as a biosensor.

As discussed above, the present invention is characterized by identifying a specific oxidation peak potential in a low potential region, which cannot be obtained by the tetrazolium salt used in Burd employing an electrochemical measuring method, and providing a biosensor. Moreover, formazan as used in the present invention is water-soluble. Thus, the formazan does not require treatment such as dissolution, and enables measurement to be made conveniently in a short time.

In items 8-9 of the Official Action, original claims 1-3 are rejected as being unpatentable under 35 U.S.C. § 103(a) over two documents. Applicants respectfully disagree and request further consideration.

Applicants submit their quantification method as defined in claims 1 to 3 of the present application is not obvious over a combination of EP0908453 A1 and Burd et al.

Generally, previous studies have shown that a formazan resulting from a tetrazolium salt is a product of an irreversible reaction, and cannot be electrochemically measured (*Angewandte Chemie*, Vol. 64, p. 391 (1952)). Burd et al does not describe the results of actually measuring the oxidation peak potential of tetrazolium salts. The present inventors studied the formazan disclosed in Burd, but found no specific oxidation peak potential in the range of from -1.0 V (vs. Ag/AgCl) to $+1.0$ V (vs. Ag/AgCl). Only the use of WST-1 of the present invention was successful in finding a specific oxidation peak potential at about $+0.5$ V (Fig. 2).

The inventors of EP0908453 published a paper on WST-1 (*Analyst* January, Vol. 120, p. 113 (1995)). In this paper, they showed the results of measurements made using a cyclic voltammetry. According to the results, a potential was applied in the range of from -1.0 V to $+0.4$ V. Within this range, no specific oxidation peak potential was detected. Their view was that because the reaction involved was an irreversible reaction, two oxidation peaks were not observed.

In view of these findings, and the general theory that the formation of a formazan from a tetrazolium salt is an irreversible reaction, the inventors of EP0908453 did not envision a method for electrochemical detection. Burd et al describes a method of electrochemically measuring tetrazolium, but does not present any grounds, such as the oxidation peak potential by use of tetrazolium salts, or the results of measurement using the oxidation peak potential. In the light of these facts, a combination of EP0908453 and Burd et al does not render obvious claims 1-3 of the present application. Accordingly, the present invention is not obvious over this combination.

For the above reasons it is respectfully submitted that the claims of this application define inventive subject matter. Reconsideration and allowance are solicited.

Attached hereto is a marked-up version of the changes made to the claim(s) by the current amendment. The attached page(s) is captioned "Version With Markings To Show Changes Made."

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

The paragraph beginning at page 5, line 25 through page 6, line 10:

In the present invention, the substrate in the sample undergoes a specific enzyme reaction under the action of the dehydrogenase and the coenzyme contained in the reaction reagent to form a reduced coenzyme. Then a redox reaction quickly proceeds between this reduced coenzyme and the electron mediator and the tetrazolium salt, and a chemically stable formazan is formed as the final product. As the above mentioned series of reactions proceed, formazan is produced depending on the concentration of the substrate. Next, the formazan is electrochemically changed by applying a potential to the electrode system and the thus arising response current is detected. Since this response current occurring from the formazan depends on the substrate concentration, the substrate can be thus quantified. Fig. 5 roughly shows the process of a series of reactions as described above. Fig. 6 shows the fundamental structural formulae of the tetrazolium salt [reacting finally] and the formazan formed as the final product.

IN THE CLAIMS

1. (Amended) A method of quantifying a substrate in a sample which method comprises the steps of:

(a) contacting a sample containing a substrate with [performing an enzyme reaction and a redox reaction between] a reaction reagent comprising at least a dehydrogenase, a coenzyme, an electron mediator and a tetrazolium salt performing an

enzyme reaction and a redox reaction between which produces a water-soluble, stable formazan, then [and the sample], [and]

- (b) detecting [a] the water-soluble formazan formed as the final reaction product
- [by] using an electrode system made of electrically conductive materials, and
- (c) correlating an increase in electrical current in the electrode system with a quantity of substrate in the sample.